Overview of Anti-Tuberculosis (TB) Drugs and Their Resistance Mechanisms

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Abstract: One-third of the world's population is infected with *Mycobacterium (M.) tuberculosis*. Tuberculosis continues to be the most common infectious cause of death and still has a serious impact, medically, socially and financially. Multidrug-resistant tuberculosis (MDR-TB), caused by tubercle bacilli that are resistant to at least isoniazid and rifampin, is among the most worrisome elements of the pandemic of antibiotic resistance because TB patients for whom treatment has failed have a high risk of death.

Drugs used to treat tuberculosis are classified into first-line and second-line agents. First-line essential anti-tuberculosis agents are the most effective, and are a necessary component of any short-course therapeutic regimen. The drugs in this category are isoniazid, rifampin, ethambutol, pyrazinamide and streptomycin. Second-line anti-tuberculosis drugs are clinically much less effective than first-line agents and elicit severe reactions much more frequently. These drugs include *para*-aminosalicylic acid (PAS), ethionamide, cycloserine, amikacin and capreomycin. New drugs, which are yet to be assigned to the above categories, include rifapentine, levofloxacin, gatifloxacin and moxifloxacin. Recently there has been much development in the molecular pharmacology of anti-tuberculosis drugs. This review summarizes information for isoniazid, rifampicin, ethambutol, pyrazinamide, and fluoroquinolones, and describes their resistance mechanisms.

ISONIAZID

Isoniazid [isonicotinic acid hydrazide (INH)] is the most widely used of all anti-tuberculosis (anti-TB) drugs. INH was first synthesized from ethyl isonicotinate and hydrazine in 1912, but it was not until 1952 that its anti-TB activity was recognized. The first clinical study of INH was conducted in 1952 at Sea View Hospital in New York; the trial was so successful that the patients were reported to be overjoyed and "dancing in the wards" [1]. The discovery of INH represented a major milestone in the conquest of tuberculosis.

Chemistry and structure-activity relationship: INH has a simple structure consisting of a pyridine ring and a hydrazide group (Fig. **1**). INH is a colorless or white crystalline powder with a chemical formula of $C_6H_7N_3O$ and a molecular weight of 137.14. It is readily soluble in water, up to 140 mg/ml at 25 °C, but is less soluble in organic solvents. Both the pyridine and hydrazide moieties are essential for the high level of activity of INH against *M. tuberculosis*. Various derivatives of INH are either less active or have less favorable pharmacologic properties than INH [2].

Anti-TB activity: *M. tuberculosis* is highly susceptible to INH with MIC values in the range of 0.01 to 0.25 μg/ml. *M. avium* is less susceptible, with an MIC in the range of 10 to 100 μg/ml. INH is active against growing tubercle bacilli but not resting bacilli. Oxygen plays an important role in INH action, probably because of the oxygen requirement for KatG-mediated INH activation [3]. INH has no activity against *M. tuberculosis* under anaerobic conditions, presumably because KatG-mediated INH activation is suppressed without oxygen. Structurally related organic acid hydrazides such as benzoic acid hydrazide and nicotinic acid hydrazide reduce INH activity. This antagonism is likely a result of competition for KatG-mediated drug activation and concomitant inactivation of the KatG enzyme by the related hydrazide [4]. INH at 10 mg/kg is highly active against *M. tuberculosis* in various animal models, such as guinea pigs, mice and monkeys [5]. In the mouse model of TB infection, bacterial colony counts in infected organs decrease quickly in the first few days of INH treatment. However, the early bactericidal activity of INH becomes slower after the first 2 to 3 weeks in mice. The number of colony-forming units stabilizes at a low level after 2 to 3 months of treatment with INH. Longer treatment with INH monotherapy may lead to the emergence of INH-resistant organisms and the number of colony-forming units in the infected organs may increase again.

Mechanism of action: Despite the seemingly simple structure of INH, its mode of action is one of the most complex of all antibiotics because it interferes with nearly every metabolic pathway in *M. tuberculosis*. The current model is: INH enters tubercle bacilli by passive diffusion and is activated by KatG to a range of reactive species or radicals and isonicotinic acid. These reactive species and radicals include both reactive oxygen species and organic radicals. These species and radicals then attack multiple targets, e.g., mycolic acid synthesis (InhA, KasA), DNA damage, lipid peroxidation, and nicotinamide adenine dinucleotide (NAD) metabolism in the cell. Deficient efflux (EfpA) and insufficient antagonism (AhpC) of INH-derived radicals as a result of defective antioxidative defenses may underlie the unique susceptibility of *M. tuberculosis* to INH.

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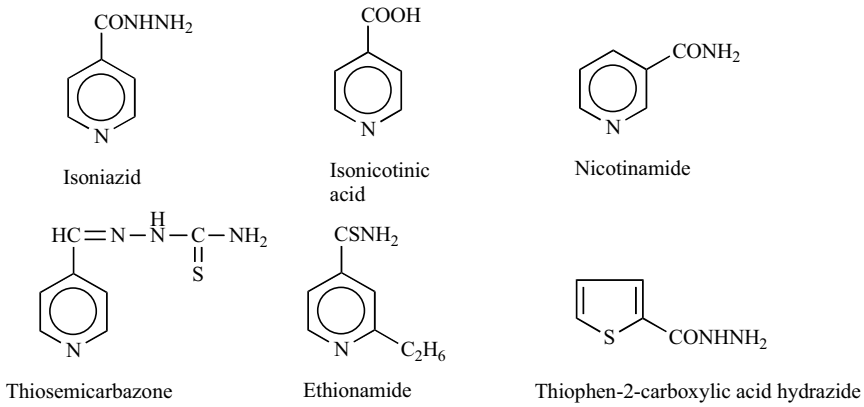

Fig. (1). Structure of isoniazid and related compounds.

INH enters *M. tuberculosis* through diffusion [6]. It is a prodrug that has to be activated by the *M. tuberculosis* catalase-peroxidase enzyme (KatG) encoded by the *katG* gene [7] to generate a range of highly reactive species for bactericidal activity. *M. tuberculosis* KatG is a bifunctional enzyme with a molecular weight of 80 kDa that has both catalase and peroxidase activity [7,8]. The native KatG enzyme is a dimer that contains one heme moiety per subunit [9]. The process of INH activation is not well understood. The availability of cloned KatG has stimulated a great deal of recent interest in understanding the mechanism of INH activation by KatG [10,11]. Manganese was shown to enhance KatG-mediated INH activation and production of the InhA inhibitor isonicotinic acyl-NADH (reduced form) [12]. The radicals produced by KatG-mediated INH activation include both reactive oxygen species such as superoxide, peroxide, and hydroxyl radical and reactive organic species such as isonicotinic acyl radical or anion, some electrophilic species, and acylperoxy and pyridyl radical adducts [13-15]. These KatG-derived reactive species and radicals affect multiple cellular targets and are associated with the bactericidal activity of INH. INH has been shown to cause accumulation of soluble carbohydrates and phosphate esters as well as inhibition of mycolic acid synthesis, phospholipid synthesis, DNA synthesis, and protein synthesis. The primary target of INH inhibition is thought to be the mycolic acid synthesis pathway [16], as reflected by the loss of acid-fastness in mycobacteria after INH treatment. Indeed, the InhA enzyme [enoyl acyl carrier protein (ACP) reductase], which is involved in elongation of fatty acids in mycolic acid synthesis, has been identified as a molecular target for INH inhibition [17]. A series of biochemical and crystallographic studies over the past few years has shed much light on the mechanism by which INH inhibits InhA [18, 19]. INH does not bind or inhibit InhA in the absence of KatG but does so after the addition of KatG, indicating that inhibition of InhA by INH is KatG-dependent. Another enzyme, KasA (ketoacyl ACP synthase), involved in meromycolate extension of mycolic acid synthesis, has been implicated by its ability to bind radioactive INH. InhA is proposed to be a target for INH inhibition [21]. The molecular basis of KasA inhibition by INH is not yet known. In addition, INH has been proposed to interfere with NAD metabolism by being incorporated into NAD to form pseudo-NAD, which cannot function as an electron acceptor in the respiratory chain. This can, in turn, affect various functions of NAD such as energy metabolism and DNA repair, in which NAD is a cofactor for the DNA ligase needed for the repair of nicked DNA [22]. It is important to note that INH has multiple effects on tubercle bacilli, and it is not always easy to pinpoint which event comes first and which is the essential target whose inhibition leads to cell death, making it necessary to evaluate the relative importance of the aforementioned factors in INH action. Wilson *et al*. [23] used microarray technology to examine the genes induced by INH treatment in *M. tuberculosis*. Genes encoding FasII enzymes AcpM, KasA, KasB, FabD (malonyl-coenzyme A-malonyl ACP transacylase), AccD (acetyl-coenzyme A carboxylase ß chain) were induced. The trehalose dimycolyltransferase C (antigen 85 C), involved in mycolate transfer, was also induced. The *ahpC* gene encoding the antioxidant enzyme hydroperoxide reductase, which is overexpressed in KatG-negative INH-resistant strains in compensation for loss of KatG [24], was also up-regulated. Four genes of unknown function, Rv1592c, Rv1772, Rv0341 and Rv0342, were induced. However, the *inhA* gene, which encodes a target for INH, was not induced by INH.

Mechanism of resistance: A great deal of progress has been made in recent years on the molecular basis of INH resistance. Mutations in *katG, inhA, kasA, ndh, ahpC* are associated with INH resistance. INH is a prodrug that requires activation by the catalase-peroxidase enzyme encoded by the *katG* gene [25]. Activated INH appears to disrupt the synthesis of essential mycolic acids by inhibiting the NADH-dependent enoyl-ACP reductase enzyme encoded by *inhA* [26]. INH resistance is likely to arise through multiple molecular mechanisms, only a subset of which has been fully characterized. Between 40 and 95% of INH-resistant clinical *M. tuberculosis* isolates have mutations in *katG*, 75 to 90% of which are located in other *katG* loci [27, 28]. Mutations in *katG*315 may be favored because mutations at this location appear to decrease INH activation without abolishing catalase-peroxidase activity, a potential virulence factor [29, 30]. *M. tuberculosis* may compensate for *katG* mutations by overexpressing the *ahpC* gene [31, 32]. INH resistance can also develop through alterations or overexpression of the INH drug target InhA, and 0 to 5% of INH-resistant *M. tuberculosis* isolates have mutations in the *inhA* open reading frame (ORF), while 8 to 20% have mutations in the *inhA*

promoter [33]. Mutations in *ndh*, a gene encoding an NADH dehydrogenase, were found to confer resistance to INH and ethionamide in *M. bovis* [34]. The *ndh* mutants had altered NADH/NAD ratios, which appeared to protect them from INH-mediated toxicity. Mutations in at least 16 other genes have been reported to be associated with INH resistance in clinical isolates [35]. However, the roles of these genes in INH resistance (if any) remain unclear. Furthermore, approximately 10 to 25% of INH-resistant strains do not contain mutations in any known gene targets for INH resistance. These results demonstrate the need for further investigations in this field. Recently, Alland and colleagues [36] presented the first large-scale analysis of 240 alleles previously associated with isoniazid resistance in a diverse set of 608 isoniazid-susceptible and 403 isoniazid-resistant clinical *M. tuberculosis* isolates. Mutations in *katG, ahpC* and *inhA* were strongly associated with isoniazid susceptibility. Remarkably, the distribution of INH resistance-associated mutations was different in INH-monoresistant isolates and multidrug-resistant isolates. Mutations in *katG*315 were significantly more common in the multidrug-resistant isolates. Conversely, mutations in the *inhA* promoter were significantly more common in INH-monoresistant isolates. Interactions among mutations and resistance to different drugs were investigated. Mutations in *katG, ahpC*, and *inhA* were associated with rifampin resistance, but only *katG*315 mutations were associated with ethambutol resistance. Genetic polymorphism associated with INH resistance is shown in Table **1**.

RIFAMYCINS

The rifamycin antibiotics are among the most potent antituberculosis agents known. Their discovery and widespread application revolutionized TB treatment, making available worldwide the use of highly effective regimens requiring only 6 months of treatment.

Rifamycins were first isolated and developed by the Lepetit Research Laboratories (Milan, Italy) [37]. The rifamycins possess a unique ansa structure consisting of an aromatic nucleus linked on both sides by an aliphatic bridge (Fig. **2**). Significant structural change at C-21, C-23, C-8, or C-1 results in markedly decreased microbiologic activity. The spatial relationships of these four sites must be maintained for antimycobacterial activity. Modifications to the side chains at C-3 are feasible without loss of activity and give rise to the three rifamycins currently in clinical use. Rifampin (or rifampicin) is a 3-formyl derivative of rifamycin S. Rifabutin is a spiropiperidyl derivative of rifamycin S. Rifapentine is cyclopentyl-substituted rifampin.

Rifamycins are bactericidal both against tubercle bacilli growing in log phase and against those in stationary phase. Studies of early bactericidal activity have demonstrated significant activity in the earlier phases of human therapy, when bacilli are multiplying most rapidly (although the activity of rifampin in this model is significantly lower than that of INH during the first 2 days of therapy [38,39]). Rifamycins are the most potent sterilizing agents used in the chemotherapy of TB. In contrast to INH, they continue to play an important role throughout the course of chemotherapy, killing tubercle bacilli for months after the start of therapy. These differences are believed to stem from the different populations of organisms thought to be present at various times and sites during therapy [40]. Again, in contrast to INH, the rifamycins begin to act quite rapidly after bacilli are exposed [41].

Rifamycins share a common primary mechanism of action. They bind to and inhibit the action of the DNA-dependent RNA polymerase of mycobacteria. The function of this enzyme is to read a DNA sequence and catalyze the polymerization of the complementary RNA chain. The enzyme is composed of four subunits, to one of which (the 1,400-amino-acid ß subunit) rifamycins bind. This binding blocks elongation of the growing RNA chain [42]. Resistance to rifamycins is most commonly conferred by the occurrence of single mutations in the RNA polymerase ß subunit gene (*rpoB*). Approximately 95% of rifampicin-resistant strains carry mutations within the rifampicin resistance-determining region (RRDR), an 81-bp region carrying codons 507 through 533 of the *rpoB* gene [43-45]. The mutation profile is shown in Fig. (**3**).

ETHAMBUTOL

EMB [dextro-2,2-(ethylenediimino)-di-1-butanol], a synthetic compound with structural similarity to D-arabinose (Fig. **4**), was first introduced in 1961. The spectrum of activity of EMB includes *M. tuberculosis* and many of the slow-growing non-tuberculous mycobacteria. The precise mode of action of EMB and the molecular basis of resistance are not fully understood. Early studies conducted with EMB demonstrated that S,S absolute stereochemistry is essential for activity and its bacteriostatic property. The effects of EMB are pleiotropic, and several hypotheses have been proposed for its mode of action. Although many of the original studies were carried out on mycobacterial species other than *M. tuberculosis*, structure-activity studies show that the effects attributed to EMB include inhibition of (a) RNA metabolism [46, 47], (b) phospholipid synthesis [48], (c) transfer of mycolic acids to cell wall-linked arabinogalactan [49], (d) spermidine synthesis [50], and (e) an early step of glucose conversion into the constituent monosaccharides of cell wall polysaccharides such as arabinogalactan and arabinomannan. EMB could conceivably behave as an arabinose mimetic, competitively inhibiting specific steps in the biosynthesis of cell wall components. The inhibitory effects of EMB on the biosynthesis of arabinan have been demonstrated at several levels [51-53]. During the course of these biochemical studies, genes encoding the cellular target of EMB were identified by using drug resistance and overexpression as a selection tool. The *embCAB* gene cluster was initially identified in an EMB-resistant strain of *M. smegmatis* and was subsequently characterized in *M. tuberculosis* [54]. These genes are likely to be organized as an operon in the order *embC, embA* and *embB*. The EmbCAB proteins are predicted to be typical membrane proteins with 12 transmembrane domains and a C-terminal globular region of approximately 400 amino acids with predicted periplasmic location. It has also been shown that EmbB is the primary target of EMB. Telenti *et al*. [54] postulated that amino acid 306 of EmbB, mutation of which may lead to resistance, is located in a cytoplasmic loop that forms an EMB resistance-determining region. Furthermore, it has been shown that amino acids in this region are well conserved among EmbB proteins across mycobacte-

Table 1. Genetic Polymorphism Associated with INH Resistance

Region	Amino Acid or Nucleotide	Comment
	Complete or partial deletion	
	M1A T11A T12P N35E A61T	Complete or partial
	D63E A65ins I71N N88R D94A	deletion of katG is
	G99E H108E,Q A110V G120del A122del	a relatively rare
KatG	G123del G125ins M126I N138S,H	event that results
	A139P S140N,A D142A L148A l150A	in high-level INH
	Y155S S160L A172T T180C W198stop	resistance
	V200stop E217del F252L T262R P275T	Mutation of S315 is
	E289del W300G,stop S302R	the most prevalent
	S315T,N,I,R,G	mechanism of resistance
	W328L,C I334T I335T L336R	to INH.
	A350S I393N	L463R represents a
	L463R	natural polymorphism
	W477stop G485V D511del D513del	with no association
	R515C L521del Q525P F567S A574V L587M,P G593D L617del L619P G629S L634F S700P V710V A714P A717P D735A	with INH resistance
Promoter	-8t→g,a -15c→t	Numeration based on
mabA inhA	-16a→g -17g→t	nucleotide position
	-24g→t -55a	relative to mabA start codon
inhA	I16T I21V,T I47T V78A	Two- to 12-fold increase
	I95P S94A	in Km for NADH
Intergenic	-55a→g -46g→a -46 ins a,t	Numbering based on nucleotide
region	-45c→t -44t→a -42t→c	position relative to the
oxyR-ahpC	-39c→t -34→a,c -32g→a	putative transcription
	-30c→t -15c→t -12c→t	start site. A role for
	-10c→a,t -9g→a	ahpC promoter
	-6g→a -4a→g	mutation in resistance
	+4c→t +33g→a	has not been established
pseudogene	18g→a 27g→t	Polymorphisms without known relation
oxyR	28c→a	to INH resistance.
AhpC	P2S	Unclear role
KasA	D66N R121K G269S	Unclear role. G269S and G312S may be
	G312S G387D F413L	natural polymorphisms
Ndh	T110A R268H	Mutation causes increased NADH/NAD ratio which leads to INH resistance

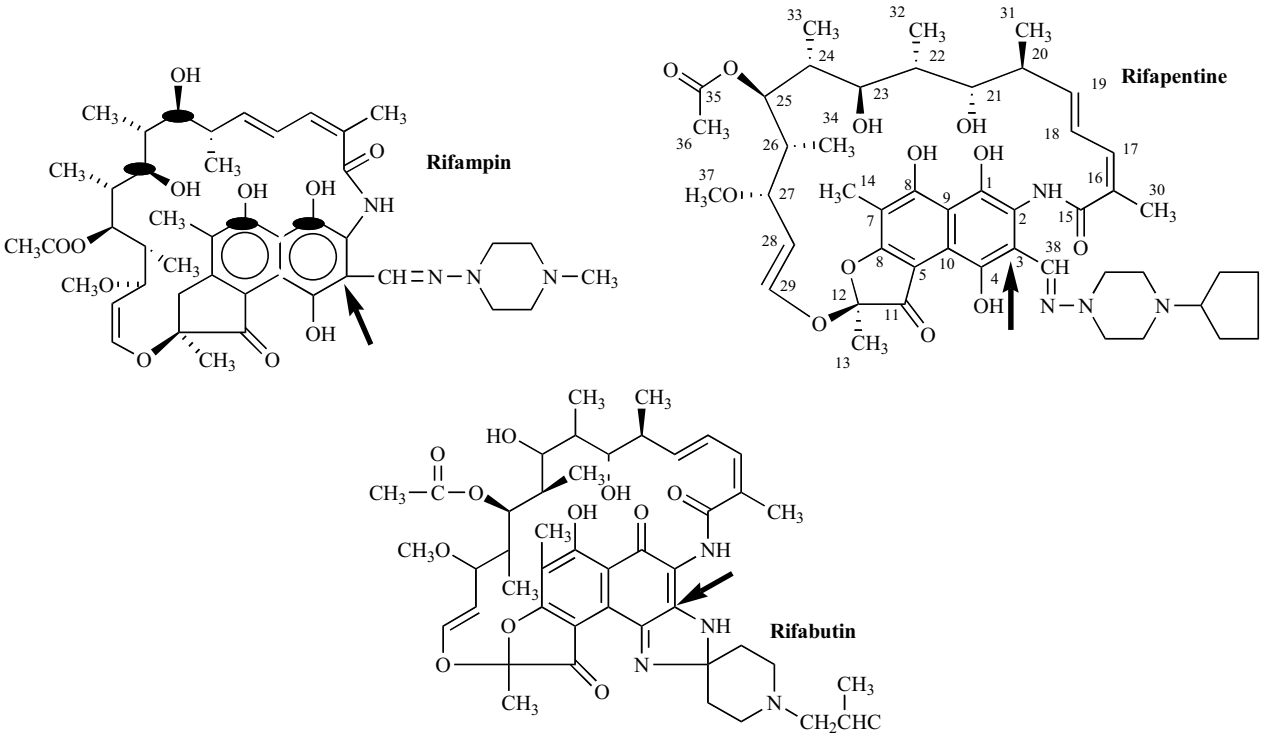

Fig. (2). Structures of three rifamycins.

rial species [55]. This implies that natural resistance to EMB results from an accumulation of genetic events determining overexpression of the Emb proteins (EmbA, EmbB, and EmbC) and/or structural mutations in EmbB [56].

Drug-resistance mechanism: EMB appears to inhibit *M. tuberculosis* by blocking the synthesis of arabinogalactan. Arabinogalactan biosynthesis is dependent on the activity of the *embABC* gene cluster, which encodes the arabinotransferases that mediate the polymerization of arabinose into arabinan. Several lines of evidence suggest that EMB exerts its toxic effect on mycobacteria by inhibiting *embABC*-encoded proteins [54], and mutations in *embABC* also appear to play a key role in the development of EMB resistance in both *M. tuberculosis* and *M. smegmatis* [57]. Associations between EMB resistance and mutations in *embA, embB* and *embC* have been reported in clinical strains of *M. tuberculosis* [58] and mutations in codon 306 of the *embB* gene (*embB*306) in *M. tuberculosis* are seen in approximately 50% of all EMB-resistant clinical isolates. In the six *embB*306 nucleotide polymorphisms that have been described, the wild-type methionine is changed to either isoleucine, leucine, valine, or threonine [59]. Each mutation is associated with an 8- to 16-fold increase in the MIC of EMB. The association

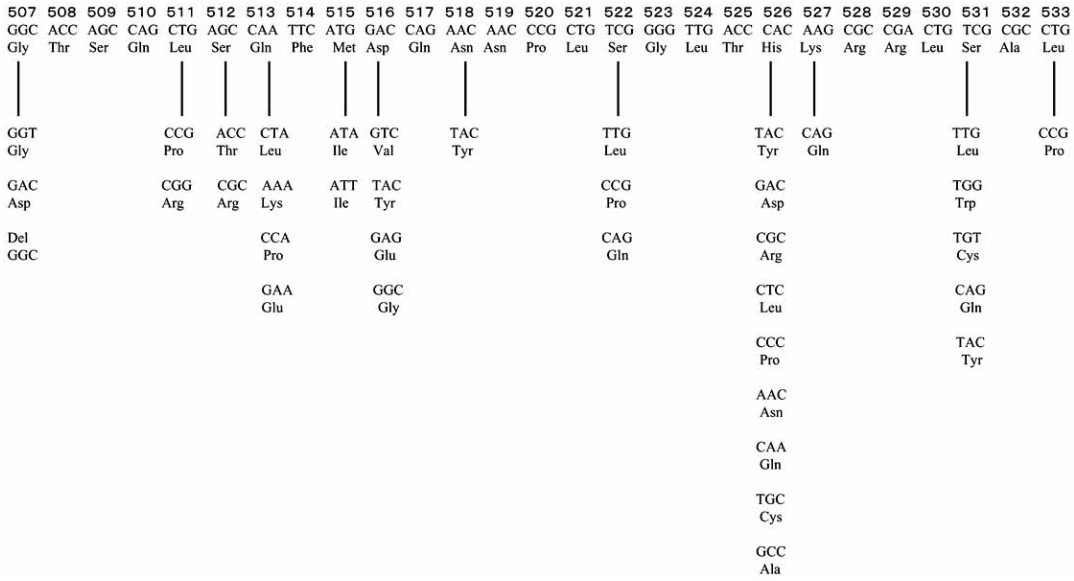

Fig. (3). Mutations located in the RRDR of M. tuberculosis rpoB gene.

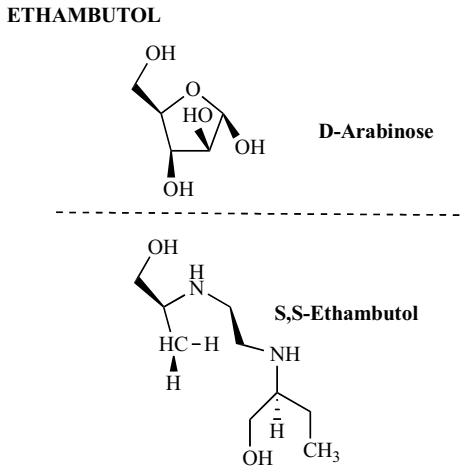

Fig. (4). Structures of ethambutol and D-arabinose.

between *embB*306 mutations and EMB resistance in clinical *M. tuberculosis* isolates is so strong that it has been proposed as a marker for EMB resistance in diagnostic tests [60]. Thus, it was a surprise when Mokrousov *et al.* first described 48 clinical *M. tuberculosis* isolates from Russia that were susceptible to EMB yet that had mutations in *embB*306 [61]. All of these EMB-susceptible *embB*306 mutants were resistant to at least one antibiotic. Several other studies confirmed these findings [62]. Recently, Alland and colleagues [63] tested 1020 clinical *M. tuberculosis* isolates with different drug susceptibility patterns and of different geographical origins for associations between *embB*306 mutations, drug resistance patterns, and major genetic group. They found that *embB*306 mutations do not cause classical ethambutol resistance but may predispose *M. tuberculosis* isolates to the development of resistance to increasing numbers of antibiotics, and additionally may increase the ability of drug-resistant isolates to be transmitted between subjects. Therefore, the mechanism of resistance to ethambutol remains unclear.

PYRAZINAMIDE

Pyrazinamide (PZA) is an important first-line drug used for the treatment of tuberculosis. It has remarkable sterilising activity, and when added to regimens containing rifampicin it is responsible for much of the killing of persisting tubercle bacilli during the initial intensive phase of chemotherapy, allowing the treatment to be shortened from 9 months to 6 months. PZA (Fig. **5**) was first synthesized by Dalmer and Walter in 1936 and was discovered as an anti-tuberculosis drug in 1952. PZA has a formula of $C_5H_5N_3O$ with a molecular weight of 123.1. PZA is active only against *M. tuberculosis*complex organisms (*M. tuberculosis*, *M. africanum*

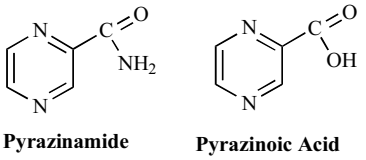

Fig. (5). Structures of pyrazinamide and its metabolite, pyrazinoic acid.

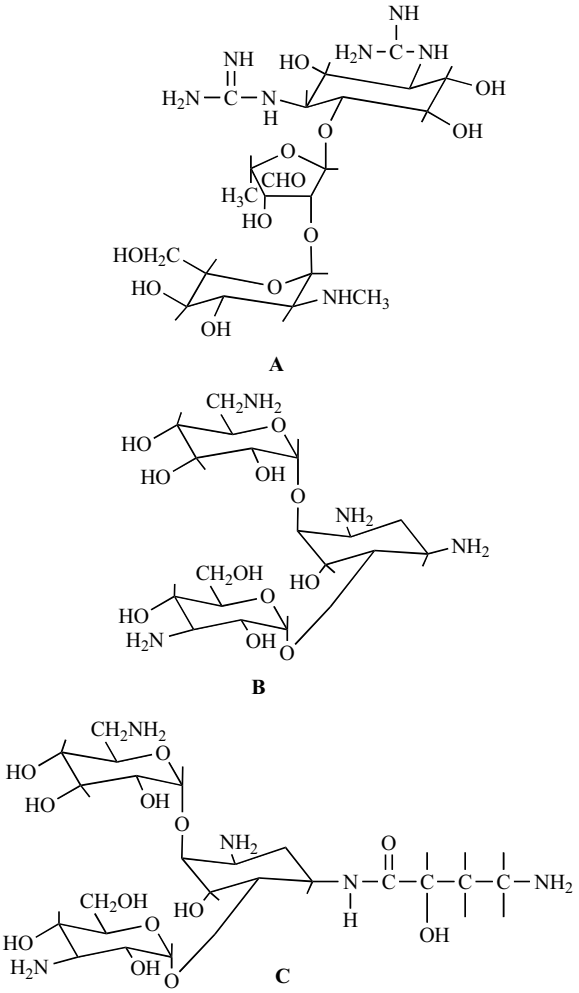

Fig. (6). Structures of streptomycin **(A)**, kanamycin **(B)**, and amikacin **(C)**.

and *M. microti*), but not against *M. bovis*. The activity of PZA is greatly affected by pH. Based on the assumption that the MIC of PZA at pH 5.5 is 50 μg/ml, the MIC values at pH 5.8 and 6.1 are 100 and 200 μg/ml, respectively. Despite the importance of PZA in the treatment of TB, its mechanism of action is probably the least understood of all anti-tuberculosis drugs. PZA is a prodrug that has to be converted to the active derivative POA by bacterial Pzase for activity against *M. tuberculosis*. Pyrazinoic acid (POA) is initially formed in the neutral cytoplasmic environment as the anion POA, which has no antibacterial activity. It is then excreted and, in acid conditions, converted in part to the uncharged protonated POA (HPOA), which enters the cell, accumulates and kills the bacterial cell. PZA-resistant clinical isolates of *M. tuberculosis* are usually defective in Pzase activity, and there is good correlation between PZA resistance and loss of this enzyme activity [64]. It is known that *pncA* mutations are the major mechanism of PZA resistance [65-67]. Identified *pncA* mutations are largely missense, causing amino acid substitutions, but in some cases are nucleotide insertions or deletions (nonsense mutations) in the *pncA* structural gene or in the putative promoter region of *pncA*. The *pncA* mutations are highly diverse and scattered along the gene. However, some degree of clustering does appear to occur at three

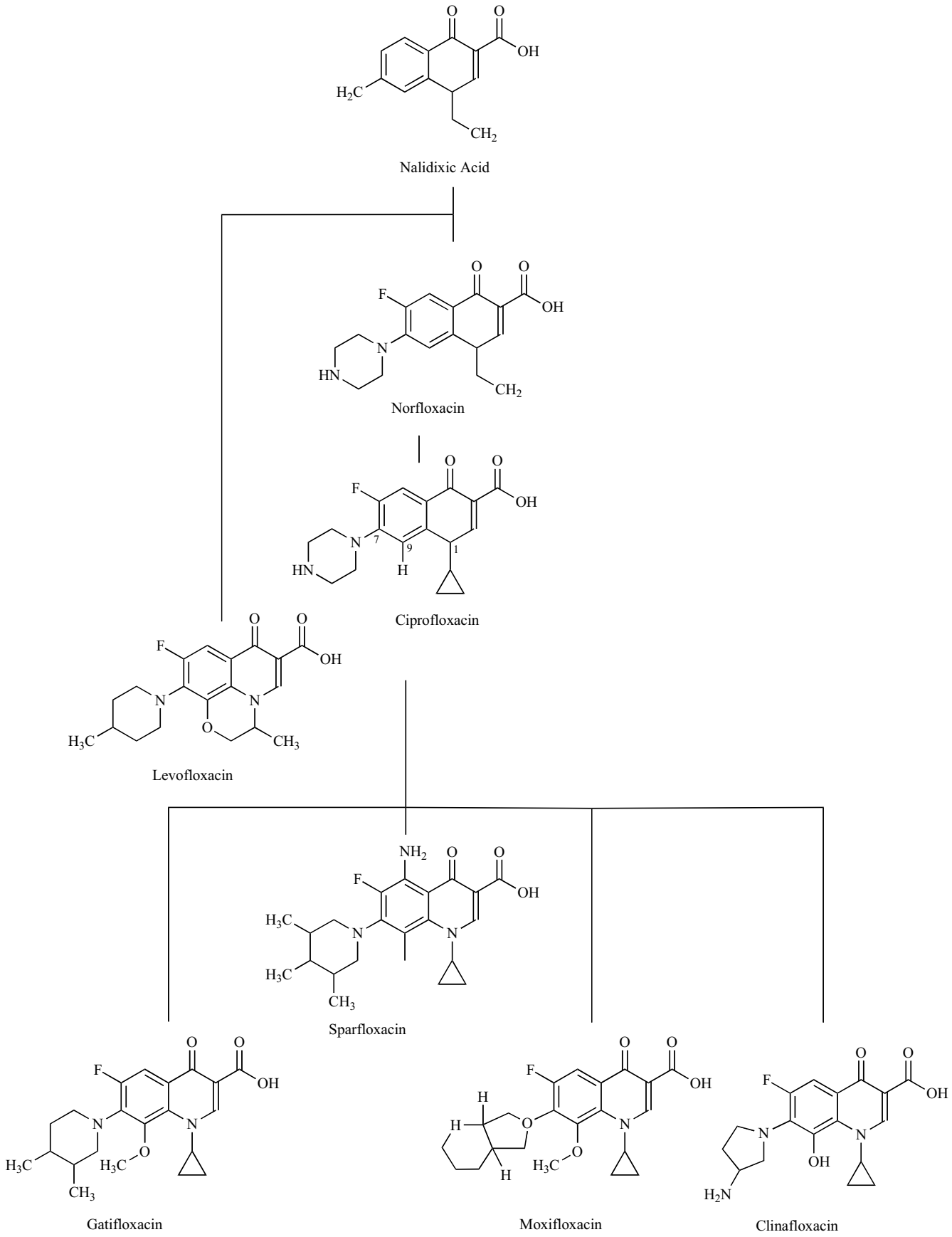

Fig. (7). Structures of fluoroquinolones.

regions of *pncA*, 3-17, 61-85, and 132-142 [68]. The high diversity of *pncA* mutations is unique to PZA resistance, since mutations in other drug resistance genes do not usually show so much diversity. Although the basis for this is unclear, it is likely that since Pzase is a non-essential enzyme there is no selective pressure on the type of mutations that can occur, and thus all types of mutations in the *pncA* gene are tolerated.

STREPTOMYCIN

Streptomycin, an aminocyclitol glycoside antibiotic, was the first antibiotic shown to be active against *M. tuberculosis*, and it was used as a first-line anti-tuberculosis drug in control programs for many years [69]. However, as a result of significant levels of drug resistance when it was used in monotherapy, the need for parenteral administration, the existence of significant side effects, and above all the availability of better drugs, streptomycin usage has declined greatly in industrialized countries since the 1960s. Now it is often replaced by ethambutol if a fourth drug is desired in addition to isoniazid, rifampicin and pyrazinamide during the initial phase of therapy. In some developing countries, streptomycin is still recommended during the initial 2 months of chemotherapy. Streptomycin acts on ribosomes and causes misreading of the genetic code, inhibition of initiation of translation of mRNA, and aberrant proofreading [70]. Resistance to streptomycin has been studied intensively in many bacteria and plants and has been shown to result from a limited number of missense mutations in the *rpsL* gene, which encodes the ribosomal protein S12 [71, 72]. Additional mutations conferring streptomycin resistance have also been found in the 16s rRNA gene *rrs*, and these mutations affect the conserved regions around nucleotides 530 and 912 [73, 74].

FLUOROQUINOLONES

Fluoroquinolones, including ofloxacin (Fig. **7**), levofloxacin, sparfloxacin, gatifloxacin and moxifloxacin, are effective drugs which are presently used to treat tuberculosis primarily in cases involving resistance or intolerance to first-line anti-tuberculosis therapy. These drugs are potential first-line agents and are under study for this indication [75]. However, fluoroquinolone resistance among *M. tuberculosis* strains is emerging, with important implications for treatment [76]. For *M. tuberculosis*, the principal target of the quinolones is DNA gyrase, a DNA topoisomerase that is composed of two A and two B subunits encoded by *gyrA* and *gyrB*, respectively. Mutations in the quinolone resistance-determining regions (QRDRs) of *gyrA* have been found to play the most important role in quinolone resistance. The nucleotide sequence of, and missense mutations within, the QRDR of *gyrA* are shown in Fig. (**8**). Codon 95 contains a naturally occurring polymorphism.

Very recently, extensively drug-resistant tuberculosis (XDR-TB) has been proposed as a result of a global survey of an international network of laboratories conducted in 2005 by the WHO. XDR-TB is defined as cases of persons with TB whose *M. tuberculosis* clinical isolates are resistant to isoniazid and rifampicin (referred to as MDR-TB) and at least three of the six main classes of second-line drugs (aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine, and *para*-aminosalicyclic acid) [77]. The use of fluoroquinolone derivatives is related to the emergence of this XDR-TB. Thus, we have to understand the medicinal chemistry of anti-TB drugs in more detail.

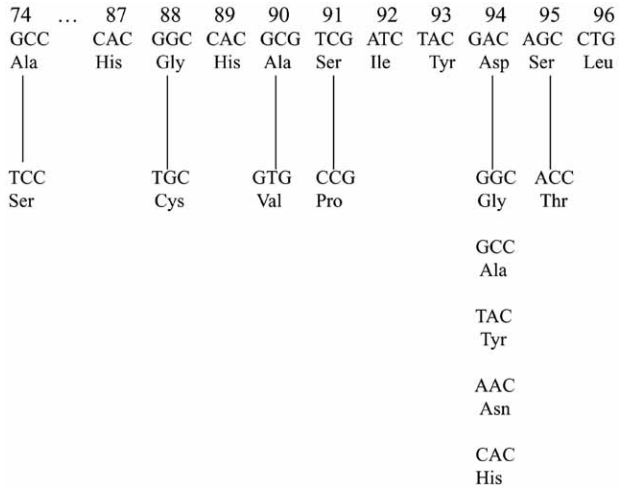

Fig. (8). Nucleotide sequence and missense mutations within the QRDR of *gyrA*.

SUMMARY

We have described the medicinal chemistry of major anti-TB drugs and the mechanisms of resistance to them. Drug-resistant tuberculosis poses a significant threat to human health. Within the last 15 years, the mechanism of action of most of the anti-tuberculosis agents has been described and we are beginning to understand some of the molecular mechanisms by which *M. tuberculosis* becomes resistant. The molecular mechanisms of resistance to isoniazid, rifampicin, ethambutol, pyrazinamide, and fluoroquinolones have been summarized here. However, the genetic basis of resistance for some anti-tuberculosis agents is not fully known. For example, the role of *oxyR, kasA,* and *ndh* gene mutations in isoniazid resistance is unclear, and the role of *embB* mutations in ethambutol resistance remains to be established. Streptomycin resistance emerges through mutations in *rpsL* and *rrs* that produce an alteration in the streptomycin-binding site, but such changes can be identified in little more than one-half of the resistant strains studied to date. Thus, a considerable amount of research into the mechanisms of resistance is still required. Additionally, it is necessary to explore relationships among drug-resistance genes and phenotypes; this kind of work is just beginning.

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